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Regulation of *Leishmania* populations within the host

III. MAPPING OF THE LOCUS CONTROLLING SUSCEPTIBILITY TO VISCERAL LEISHMANIASIS IN THE MOUSE

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SUMMARY

Acute susceptibility of the mouse to *Leishmania donovani* is largely determined by a single locus designated *Lsh*. Linkage between the *Lsh* locus and the Chromosome 1 marker *Id-1* was detected using several sets of recombinant inbred strains. Chromosome 1 linkage was confirmed in backcross generations using isoenzymes and a cytogenetic marker. The data indicate that the gene order is centromere–*Lsh–Id-1–In–Dip-1*. The estimated recombination frequency between *Lsh* and *Id-1* is $0{\cdot}12 \pm 0{\cdot}04$. This mapping is away from the known histocompatibility loci.

INTRODUCTION

The course of mouse infections with the intracellular protozoan parasite of macrophages *Leishmania donovani* falls into an early phase lasting 2–4 weeks and a subsequent later phase (Bradley & Kirkley, 1977). In an analysis of the early phase, the hepatic parasite burdens of twenty-five inbred mouse strains 2 weeks after intravenous infection varied between, but not within, strains. They fell into two distinct groups, susceptible (S) and resistant (R), regardless of the place of origin of the strain. Breeding studies showed that the S and R phenotypes are controlled by alleles at a single locus (or tight linkage group) with *r* incompletely dominant over *s* (Bradley, 1977). The locus was provisionally named *Lsh*.

In this study several sets of recombinant inbred (RI) mouse strains, backcross mice and a Robertsonian translocation have been used to map the locus onto Chromosome 1 of the mouse.

The genetic control of the immune response has become a major research area in the last few years, but attention has been concentrated on acquired responses to relatively defined antigens. Interest in actual resistance to infection, especially infection with bacteria and animal parasites rather than with viruses, has continued to be slight. We demonstrate here that resistance to the early stages of a protozoal infection is largely controlled by a gene unlinked to the major histocompatibility complex of the mouse. An accompanying paper (Plant & Glynn, 1979) shows that genetic control of susceptibility to *Salmonella typhimurium* resides on the same chromosome. The two findings taken together suggest that innate susceptibility to infections may be more tractable to precise genetic analysis than has previously been assumed.

MATERIALS AND METHODS

Recombinant inbred mouse strains. The BXD, BXH, BXJ and HP recombinant inbred (RI) strains were derived by brother–sister inbreeding beginning with the crosses of the C57BL/6J strain with the DBA/2J, C3H/HeJ, SJL/J and AKR/J strains, respectively (Taylor, Bedigian & Meier, 1977). The LXB, 58NXL and BRX58N RI strains were similarly derived from the following crosses: C57L/J × C57BL/6J, B10.D2(58N) × C57L/J, and B10.D2(58N) × C57BR/cdJ. The relevant genotypes of the progenitor strains are shown in Table 1.

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TABLE 1. Alleles of progenitors of recombinant inbred strains for relevant Chromosome 1 loci

Strain	Loci				Generic allele symbol*
	Lsh	*Id-1*	*ln*	*Dip-1*	
AKR/J	*r*	*b*	+	*b*	A
C3H/HeJ	*r*	*a*	+	*b*	H
C57BL/6J	*s*	*a*	+	*a*	B
B10.D2(58N)	*s*	*a*	+	*a*	B
C57BR/cdJ	*r*	*b*	+	*a*	BR
C57L/J	*r*	*b*	*ln*	*a*	BL
SJL/J	*r*	*b*	+	*b*	J
DBA/2J	*r*	*b*	+	*b*	D

* In Tables 2–5 these generic symbols are used for alleles inherited from these particular progenitor strains. It is helpful to remember that the symbol 'B' in those tables is always used to represent alleles from the *Lsh*^{s}-bearing progenitor strain.

Backcross mice. (C57BL/6J × DBA/2J)F_1 mice were backcrossed to the susceptible parent C57BL/6J. Forty progeny were typed on a biopsy for *Id-1* and *Dip-1* at the Jackson Laboratory, Bar Harbor, Maine, USA, where both the RI and backcross mice were bred. *Lsh* typing was carried out on the same mice after freighting to London.

Robertsonian translocation mice. Mice homozygous for the Robertsonian translocation Rb(1.3)1 Bnr, hereafter referred to as Rb1, in which the centromeres of Chromosomes 1 and 3 have been fused to form a single large metacentric chromosome, were found to be resistant to leishmaniasis and were crossed with the *Lsh*^{s} NMRI strain. Linkage between *Lsh* and the cytogenetic marker was tested by backcrossing this F_1 generation, *Lsh*^{r} Rb1/*Lsh*^{s}+, to the homozygous recessive *Lsh*^{s}+/*Lsh*^{s}+ NMRI strain. Offspring were *Lsh* typed and direct bone marrow preparations examined for the presence of a metacentric chromosome to determine whether or not they carried the translocation.

Genetic markers. The BXD RI strains had previously been typed for the electrophoretic variants of isocitrate dehydrogenase-1 (*Id-1*) and dipeptidase-1 (*Dip-1*) (Festenstein, Bishop & Taylor, 1977). Similarly, the *Dip-1* typing of the BXH and BXJ RI strains is reported elsewhere (Rosenstreich *et al.*, 1978). Additional *Id-1* and *Dip-1* typing of RI strains and backcross mice was carried out using the same methods. Backcross mice were either heminephrectomised or partially hepatectomised under nembutal anaesthesia for isoenzyme testing, prior to being sent to London for *Lsh* typing. The other chromosome 1 marker, leaden (*ln*), is a coat colour marker.

Lsh typing. The L82 strain of Ethiopian *L. donovani*, which has been kept in continuous hamster passage in our laboratory since 1972, was used. Its history prior to that date and the mode of preparation are described by Bradley & Kirkley (1977). 10^7 amastigotes were injected intravenously into the mice in 0·2 ml of medium 199 containing 5% foetal calf serum.

All animals were killed on day 15 after infection. The preparation of liver imprints and counting of parasites were carried out as described previously (Bradley & Kirkley, 1977) and the counts expressed as Leishman-Donovan units (LDU) per liver after Actor (1960). Parasite population levels in individual experimental mice were compared against levels in inbred homozygous susceptible and resistant mice in each experiment. Counts were done on randomized coded slides. There was essentially no overlap between counts from susceptible mice and those from heterozygous and resistant animals, although the latter two groups were poorly separable from each other.

Estimation of recombination frequencies. Recombination frequencies were estimated from the RI strain data using an equation derived from Haldane & Waddington (1931) which relates the probability of fixing a recombinant genotype in an RI strain, (R), to the recombination frequency (r); $R = 4r/(1+6r)$. r is estimated by $R/(4-6R)$, where R is estimated from the RI data. The variance of the estimated r is $r(1+2r)(1+6r)^2/4n$, where n is the number of RI strains contributing to the estimate.

RESULTS

The results of typing with various RI strains for known Chromosome 1 markers are shown in Tables 2–5 Among forty-nine strains whose progenitors differed at both *Lsh* and *Id-1*, there were only fourteen recombinants between the two loci, significantly fewer than expected on the assumption of independence ($P < 0{\cdot}001$). Similarly, among forty-four strains whose progenitors differed at both *Lsh* and *Dip-1*, sixteen

TABLE 2. Segregation of Chromosome 1 markers in BXD, BXJ and HP recombinant inbred strains

Strain or progenitor strain	Loci*				
	Lsh		*Id-1*		*Dip-1*
BXD 1	D	X	B		B
2	B		B		B
3	B		B	X	D
4	B		B		
5	B		B	X	D
6	D		D		D
8	D		D	X	B
9	B		B		B
11	B		B	X	D
12	B		B		B
13	D	X	B	X	D
14	B		B		B
15	D		D		D
16†	B	X	D	X	B
17	D		D	X	B
18	D	X	B		B
19	B	X	D	X	B
20	D	X	B	X	D
21	B		B		B
22	B		B		B
23	D	X	B	X	D
24	B		B	X	D
25	D		D	X	B
27	D		D		D
28	D		D		D
29	B	X	D		D
30	D		D		D
(C57BL/6J)	(B)		(B)		(B)
(DBA/2J)	(D)		(D)		(D)
BXJ 1	J		J		J
2	J		J		J
(C57BL/6J)	(B)		(B)		(B)
(SJL/J)	(J)		(J)		(J)
HP	A	X	B	X	A
(C57BL/6J)	(B)		(B)		(B)
AKR/J	(A)		(A)		(A)

* Symbols for alleles are as indicated in Table 1 where the genotypes of the progenitor strains are also given. Regions in which crossovers have resulted in recombination in the different RI strains are denoted by 'X'.

† The *Id-1* genotype of BXD-16 was incorrectly given as 'B' by Festenstein *et al.* (1977).

TABLE 3. Segregation of Chromosome 1 markers in BXH recombinant inbred strains

BXH strains or progenitor strains	Loci*		
	Lsh		*Dip-1*
BXH 2	H	X	B
3	B	X	H
4	H	X	B
5	H	X	B
6	H		H
7	H		H
8	H		H
9	H		H
10	H	X	B
11	B		B
12	B		B
14	H		H
18	H		H
19	H	X	B
(C57BL/6J)	(B)		(B)
(C3H/HeJ)	(H)		(H)

* Symbols as indicated in footnote to Table 2.

TABLE 4. Segregation of Chromosome 1 markers in LXB6 and 58NXL recombinant inbred strains

RI strain or progenitor strain	Loci*				
	Lsh		*Id-1*		*In*
LXB5 1	B	X	L		L
2	B		B		B
3	L		L		L
4	B		B		B
5	L		L		L
(C57L/J)	(L)		(L)		(L)
(C57BL/6J)	(B)		(B)		(B)
58NXL 1	L		L	X	B
2	B		B		B
3	B		B		
4	B, L		B		B
5	B	X	L	X	B
8	L	X	B	X	L
(C57L/J)	(L)		(L)		(L)
(B10.D2(58N))	(B)		(B)		(B)

* Symbols as indicated in footnote to Table 2.

TABLE 5. Segregation of Chromosome 1 markers in BRX58N recombinant inbred strains

BRX58N strain or progenitor strain	Loci*	
	Lsh	*Id-1*
1	BR	BR
3	BR	BR
4	BR X	B
7	B	B
8	BR	BR
9	BR	BR
10	BR X	B
11	BR	BR
12	B	B
13	BR, B	B
(C57BR/cdJ)	(BR)	(BR)
(B10.D2(58N))	(B)	(B)

* Symbols as indicated in footnote Table 2.

TABLE 6. Estimates of recombination frequencies between loci obtained from the RI data using the Haldane & Waddington (1931) equation

Region of recombination	Observed number of strains with recombinant genotypes	Estimated recombination frequency
Lsh–Id-1	14/49 (0·2857)	0·13±0·05
Id-1–Dip-1	13/30 (0·4333)	0·31±0·18
Lsh–Dip-1	16/44 (0·3636)	0·20±0·23
Lsh–In	3/11 (0·2727)	0·12±0·10
Id-1–In	3/11 (0·2727)	0·12±0·10

TABLE 7. Linkage between translocation Rb1Bnr and *Lsh*

	Metacentric	Parasite count	Number of mice	
Parents: Rb1Bnr-bearer	Present × 2	Very Low	—	
NMRI	Absent	High	—	
F_1	Present	Low	—	
Backcross	Present	Low	17	26
	Absent	High	9	
	Present	High	1	3
	Absent	Low	2	

TABLE 8. Alleles transmitted by (C57BL/6J × DBA/2J) F_1 hybrids when backcrossed to C57BL/6J

Gametes					Number of mice		Region of recombination
Lsh		*Id-1*		*Dip-1*			
s		*a*		*a*	10	27	None
r		*b*		*b*	17		
s	X	*b*		*b*	2	3	*Lsh–Id-1*
r	X	*a*		*a*	1		
s		*a*	X	*b*	3	9	*Id-1–Dip-1*
r		*b*	X	*a*	6		
r	X	*a*	X	*b*	1	1	*Lsh–Id-1, Id-1–Dip-1*
Total					40		

10% recombination between *Lsh* and *Id-1*; 25% recombination between *Id-1* and *Dip-1*; and 30% recombination between *Lsh* and *Dip-1*.

recombinants were found, and among thirty strains differing at both *Id-1* and *Dip-1*, thirteen recombinants were found. In each case, for eleven strains whose progenitors differed at both *Lsh* and *ln* and for eleven strains differing at *Id-1* and *ln*, three recombinants were present. Estimates of recombination frequencies between loci obtained from these data using the Haldane & Waddington (1931) equation appear in Table 6.

The results of the linkage test with Rb1 are given in Table 7. In the twenty-nine backcross mice tested, resistance to infection was significantly correlated with the presence of Rb1 ($P \leqslant 0{\cdot}001$). Only three crossovers were scored for an estimated frequency of recombination between *Lsh* and the centromere of $0{\cdot}10 \pm 0{\cdot}06$.

The results of a three-point backcross classified for *Lsh*, *Id-1* and *Dip-1* (Table 8) confirm linkage between *Lsh* and *Id-1* ($P \leqslant 0{\cdot}0001$) and establish the gene order *Lsh–Id-1–Dip-1*. Among the forty mice tested there were three single crossovers between *Lsh* and *Id-1*, nine single crossovers between *Id-1* and *Dip-1*, and one double crossover. The alternative gene order, *Id-1–Lsh–Dip-1*, necessitates postulating three double crossovers. On the basis of these backcross data, the estimated recombination frequencies between *Lsh* and *Id-1*, and *Id-1* and *Dip-1*, are $0{\cdot}10 \pm 0{\cdot}05$ and $0{\cdot}25 \pm 0{\cdot}07$, respectively. Twelve of the forty backcross mice, including one *Lsh–Id-1* single crossover, three *Id-1–Dip-1* single crossovers and the double crossover, were produced by mating hybrid males to C57BL/6J females. The other twenty-eight mice were from hybrid females mated to C57BL/6J males.

DISCUSSION

Presumptive evidence that *Lsh* is linked to the Chromosome 1 marker *Id-1* was provided by the RI strain data. This linkage was supported by the cytogenetic demonstration of linkage between *Lsh* and the Robertsonian translocation Rb1Bnr, which is a marker for the centromeres of chromosomes 1 and 3. Linkage was confirmed by a three-point backcross involving *Id-1* and *Dip-1*. While the RI data are consistent with either a proximal or a distal placement of *Lsh* relative to *Id-1*, the backcross data clearly favour the proximal position, indicating the following gene order: centromere–*Lsh–Id–1–ln–Dip–1* The frequency of recombination between *Lsh* and *Id-1* is estimated to be $0{\cdot}13 \pm 0{\cdot}05$ from the RI data, which is in excellent agreement with the estimate obtained from the backcross data, $0{\cdot}10 \pm 0{\cdot}04$. The weighted average of the two estimates is $0{\cdot}12 \pm 0{\cdot}04$. Since double crossovers are rare in short intervals we can equate map distance with recombination frequency and assign the *Lsh* locus to a position approximately 12 centimorgans proximal to *Id-1* on Chromosome 1.

In the linkage test of *Lsh* with the Rb1 metacentric chromosome, only three recombinant mice were scored among a total of twenty-nine backcross progeny, demonstrating linkage of the *Lsh* locus to the centromere of either Chromosome 1 or Chromosome 3 ($P \leqslant 0{\cdot}001$), a result consistent with the postulated Chromosome 1 linkage. The estimated frequency of recombination between the *Lsh* locus and the centromere, in Rb1 heterozygotes, is $0{\cdot}10 \pm 0{\cdot}16$. Since recombination between genetic markers on the proximal end of Chromosome 1 is known to be reduced in Rb1 heterozygotes (Davisson & Roderick, 1975), the present observed recombination frequency between *Lsh* and the centromere is expected to underestimate the normal recombination frequency. Nonetheless, it is of interest to compare the marker–Rb1 recombination frequency obtained here for *Lsh* ($0{\cdot}10 \pm 0{\cdot}06$) with that previously obtained for *fz* ($0{\cdot}01 \pm 0{\cdot}01$) and *ln* ($0{\cdot}33 \pm 0{\cdot}03$). The intermediate frequency obtained for *Lsh* is consistent with the assigned position of *Lsh* based on the RI and three-point cross results.

Placing a locus in the linkage map is a useful means of distinguishing it from previously described and mapped polymorphic loci. Thus we can say that *Lsh* is distinct from *H-2*, *Ig-1*, and all other mapped histocompatibility and other alloantigen-determining loci (Davisson & Roderick, 1978). A possible exception is the H(*ln*) histocompatibility locus found by Bailey (1972) to be linked to *ln*. However, the recombination frequencies he obtained with *ln* ($0{\cdot}09 \pm 0{\cdot}06$) and *fz* ($0{\cdot}30 \pm 0{\cdot}10$) would place H(*ln*) between *ln* and *Id-1*, distal to the location of *Lsh*. The *Mls* locus (Festenstein, 1976), which is the major lymphocyte-activating determinant outside the major histocompatibility complex (*H-2*), is also on Chromosome 1 but still more distally located (Festenstein *et al.*, 1977).

Although the aldehyde oxidase locus, *Aox*, lies in the same vicinity of chromosome 1 as *Lsh* (Watson *et al.*, 1972), it is unlikely that the two loci are identical. The five mouse strains with a high K_m value aldehyde oxidase include the Lsh^s DBA/1 and Lsh^r DBA/2 and CBA/J, while the majority of mouse strains having a lower K_m value includes four strains susceptible to leishmaniasis and five resistant.

The mapping, therefore, does not identify a known polymorphic locus as being responsible for acute susceptibility to leishmaniasis. If a structural gene only is involved, then the mapping excludes polymorphic loci located elsewhere from the resistance mechanism. On the other hand, the *Lsh* locus could be acting as a regulatory rather than a structural gene, in which case the mapping does not restrict the enzyme system that might be responsible for leishmaniasis resistance. At present, the mechanism by which *Lsh* affects susceptibility to leishmaniasis is unknown.

The *Lsh* gene is a clear example of a single locus having a large effect in determining acute susceptibility or resistance to an infective disease. Rather few comparable situations are known, mostly concerning viral infections. Bang has described variations in susceptibility to yellow fever virus and mouse hepatitis virus which seem to have a simple genetic basis (Bang & Warwick, 1960). Viral susceptibility studies have shown the importance of genes linked to the major histocompatibility complex (Lilly, 1966; Lonai & Haran-Ghera, 1977) and it may be expected that many responses will be related to this complex. Among the non-viral infections, susceptibility is dominated by a single locus in *Salmonella typhimurium* infection (Plant & Glynn, 1976) and experimental scrub typhus (Groves & Osterman, 1978). The present case appears to be among the first where a locus having a large effect on acute susceptibility has been mapped. It is interesting to compare our result with that of Plant & Glynn (1979) who found the determinants for susceptibility to *Salmonella typhimurium* also to be located on Chromosome 1. Their mapping does not allow a precise location of the locus but, as the spectrum of susceptibilities in their experiments corresponds to leishmaniasis susceptibility (Bradley, 1974; 1977), it is possible that their gene is identical with *Lsh*. The scrub typhus resistance gene has been mapped to another chromosome (M. G. Groves & J. V. Osterman, personal communication).

It is clear that recent advances in genetics and in immunology make the genetic analysis of host responses to infective disease tractable. Attention has been concentrated on the area of the major histocompatibility complex because of the increasing evidence of its important role in determining immune responses to defined antigens. It is also clear that there are marked *H-2* restrictions manifest in the adoptive transfer of resistance to bacteria (Zinkernagel *et al.*, 1977) as well as viruses. While this and the comparable loci in man, HLA, have been shown to affect susceptibility to several non-infective diseases (Bodmer, 1978) and to the likelihood of joint pathology complicating infections with chlamydia and the

gonococcus, their role in determining actual resistance to infecting agents has only been defined for viruses.

The present study makes it clear that genetic analysis outside the *H-2* region is also relevant to understanding innate immunity. We have given reasons elsewhere (Bradley, 1977) for not considering leishmaniasis to be a special case, and the conclusions of Plant & Glynn (1979) and Groves & Osterman (1978) confirm this. Genetic understanding of acute, or innate, immunity enables this to be standardized in experiments to analyse recovery and acquired immune responses, thereby making these more tractable, as subsequent experiments have demonstrated.

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